Hemoglobin A1c in Blood, DCA 2000+ v031216

I. Purpose:

The term @glycosylated hemoglobin@ refers collectively to a series of stable compounds that are formed between hemoglobin and sugars. Their concentrations are increased within erythrocytes of patients with diabetes mellitus. The level of hemoglobin $_{A1C}$ is proportional to the level of serum glucose over a period of two months. Thus, hemoglobin $_{A1C}$ is considered to be an indicator of the mean daily blood glucose concentration over the preceding two months.

The aim of therapy by clinicians is to maintain patient glucose levels at constant normal or near normal levels in the blood. Therefore, the measurement of this long-term blood glucose facilitates the patients ongoing therapy. Recent studies have shown that results from the hemoglobin $_{A1C}$ leads to changes in diabetes treatment and improvement of metabolic control as indicated by lowering hemoglobin A1c(HbA1c).

The DCA 2000+ uses an immunochemical method for the measurement of HbA1c. An agglutinator, present as one of the reagents, causes agglutination of specific anti-HbA1c coated latex particles. This is measured as an increase in turbidity by the spectrophotometer (the DCA 2000+).

II. Specimen:

Whole blood may be obtained from a finger prick or venipuncture. Acceptable anticoagulants are EDTA, heparin, citrate and fluoride/oxalate. Clotted specimens are unacceptable. Reject the clotted specimen and request a new sample from the client for analysis.

The provided glass capillary (within plastic capillary holder) holds 1µl of blood. This system must be used for this procedure. NOTE: Once the glass capillary is filled with the sample, analysis *must* begin within 5 minutes.

For specimen collection, refer to Appendix A.

III. Materials and Equipment:

A. Materials Supplied With Kit
   1. Capillary holders
   2. Calibration card
   3. Reagent cartridges

B. Materials not Supplied with Kit
   1. Kimwipes or gauze
   2. Disposal exam gloves latex or equivalent
   3. Biohazard sharps/waste container
   4. Disinfectant for work area
   5. QC Log Sheet
   6. Daily Patient Log Sheet
   7. DCA 2000+ Analyzer

III. SAFETY:

Wear disposable examination gloves and a lab coat for personal protective equipment. . Use universal precautions when handling all blood and blood products. Treat all specimens as if they contained an etiologic agent

IV. Quality Control:

A. A control kit that includes 2 normal and 2 abnormal blood controls can be purchased through Bayer, product number 5068. The instructions for preparation of these controls are as follows:

Reconstitution of Controls:

1. Remove the control bottle(s) from refrigerator just prior to reconstitution.
2. Gently tap the bottom of the control bottle on the counter in order to dislodge any particles from the stopper.
3. Carefully remove the cap from the bottle.
4. Holding the Reconstitution Fluid dropper bottle vertically, discard the first drop and add six (6) drops of fluid to the control bottle.
5. Carefully replace the cap and swirl the control bottle several times. Let stand at room temperature for 15 minutes.
6. Mix the control(s) by inversion until the solution is homogeneous and all lyophilized material is reconstituted.
7. Remove and discard cap and replace with the supplied eyedropper.
8. Label the bottle with the reconstitution date. This reconstituted control is stable for 3 months when kept refrigerated and tightly capped, and needs no further preparation.
9. Refer to section VI C and D for testing the reconstituted control.

B. Frequency:

1. Run a normal and abnormal at the beginning of each day of use and for each new lot number of reagent kits.
2. Thereafter, one (alternate use of normal and abnormal control) per kit of 10 reagent cartridges of the same lot number.
3. The manufacturer will establish the acceptable control range with each lot of controls purchased. If the bar code on the control card (included in the DCA 2000 HbA_{1c} Control Kit) was scanned before running the control, the instrument will automatically indicate (via the display screen) whether the control result is within or out-of-limits. Otherwise the acceptable range for that lot number is on the manufacturer=s package insert.
4. If control results are out-of-limits, do not proceed with specimen analysis. Check reagent cartridges, instrument, environmental conditions and technique. Correct any problems and then re-assay controls to verify that control results are with acceptable limits. Document all actions on the QC Log.
5. All QC data must be stored for a minimum of two (2) years.

C. DCA Optical Test

The DCA is shipped with an optical test cartridge in the cartridge compartment. Before initial use, run the optical test cartridge and make a permanent record of these results. After the

initial reading the routine frequency is as follows;

- Quarterly
- after cleaning the cartridge compartment
- after changing the air filter
- when the operator suspects the DCA is malfunctioning
- To run this cartridge, refer to page 2.17 of the operator=s manual.

V. Procedure:

A. Instrument Preparation and Calibration:

1. Calibrate the system for each new lot number of reagent cartridges.
2. Allow the instrument to warm up for 8 minutes before use.
3. Calibrate the machine as follows;
   a. Locate the dot on the DCA 2000+.
   b. Hold the control card so that the bar code faces right.
   c. Insert the card into the bar code track above the dot on the DCA 2000+. Hold the card gently against the right side of track.
   d. Quickly and smoothly, slide the card down past the dot.
   e. A beep sound to signal a successful scan, press enter. If no beep sounds, repeat the procedure.
   f. If a beep repeatedly fails to sound, refer to the Trouble Shooting section in the DCA 2000+Operator=s Manual.

B. Reagent Preparation:

1. Remove the foil pouch, which contains the test cartridge, from the refrigerator. Before opening the foil pouch, allow it sit at room temperature for a minimum of ten (10) minutes.
2. To open foil pouch, tear down from corner notch until entire side is open. Do not use scissors to cut pouch open. Scissors can damage the reagent cartridge, or the enclosed desiccant pillow.
3. Once the pouch is open, do not touch the optical window.
4. Discard the reagent cartridge if:
   - cartridge is damaged,
   - the flexible plastic pull-tab is loose or missing,
   - the desiccant is missing or,
   - loose desiccants particles are found inside the foil pouch.
5. Once the foil pouch has been open, the cartridge must be used with one (1) hour. Do not place the cartridge back in the refrigerator once it has been opened.

C. Procedure for Running Reconstituted Control

1. Use the DCA 2000+ Hemoglobin A_{1C} normal control and abnormal controls in the same manner as a patient sample.
2. Bring all reagents and controls to room temperature before use.
3. To automatically set up the DCA 2000+ Analyzer for running the control and to store the result automatically in the control memory buffer, use the DCA 2000+ CONTROL CARD found in the control kit. The control card, one side for the Normal Control and

one side for the Abnormal Control, is used in exactly the same way as the reagent calibration card. (See VI A.3)

4. Once the control bar code(s) have been read, press enter when beep sound is heard. Proceed with the controls following the same procedure as a patient sample, steps VI. D and VI. E.

D. Sample Preparation:
   1. Bring all reagents and controls to room temperature before testing.
   2. IMPORTANT: Once the glass capillary is filled with sample, analysis must begin within 5 minutes.
   3. Record the patient=s name and date of testing on the daily patient log sheet.
   4. Open a capillary holder and inspect the capillary holder for the presence of an absorbent pad, a glass capillary and a latching mechanism.
   5. Fill the capillary with blood by one of the following methods;
      a. From a finger stick; Holding the capillary holder at an angle, touch the tip of the capillary to a drop of blood on the finger until the capillary is filled.
      b. Filling the capillary with blood with venous blood; Mix the sample well by inversion and carefully remove the stopper from the blood collection tube. Hold the capillary assembly at an angle and touch the tip of the capillary to the blood sample on the stopper until the capillary is filled.
   6. Using a Kimwipe or gauze, carefully wipe the outside of the glass capillary tube. Do so in a manner that the open end of the capillary does not touch the Kimwipe.
   7. Inspect the glass capillary for any air bubbles. If bubble(s) are present, discard capillary holder; then repeat procedure using a new capillary.
   8. Carefully insert the capillary holder into the REAGENT cartridge (flat side towards cartridge) until the holder snaps into place.

E. Analyzing the Patient Sample:
   1. Using the same procedure as VI-A.3, slide the REAGENT cartridge through the bar code track.
   2. Open the cartridge compartment door.
   3. Hold the REAGENT cartridge so that the bar code faces to the right. Insert the REAGENT cartridge into the cartridge compartment until it snaps into place.
   4. Slowly remove the plastic pull tab using a continuous motion. Make sure that the pull tab is completely removed from the cartridge.
   5. Close the door and the DCA will read the sample.
   6. A result will display on the DCA 2000+ screen in approximately 6 minutes. Record this result on the Daily Patient Log Sheet.
   7. To remove the cartridge, open the door and locate the button to the right of the cartridge compartment. Push and hold down this button with your right hand. Use your left hand to gently push the plastic tab on the cartridge to the right. This should release the cartridge. Remove and discard the used cartridge.
   8. Disinfect the work area either at the end of the day, or when testing has ceased.

IV. Reporting of Results:

A. The displayed result is the patient=s percent Hemoglobin A_{1C}. Record the patient=s result as a percentage.

B. The suggested reference range (in normal healthy individuals) for the DCA 2000+ is 4.3 - 5.7%.

C. HbA_{1C} is approximately 3-6% in non-diabetics, 6-9% in controlled diabetics, and can be elevated to 20% in poorly controlled diabetics.

V. Procedure Notes:

A. Bayer has included a temperature indicator to help monitor the atmospheric conditions when the kits are being shipped. The indicator is found on the inside top lid of the kit=s container. If this indicator has turned red, do not use the kit. Indicate the date and time of receipt and call the manufacturer.

B. The reconstituted control may remain at room temperature for only 30 minutes during testing time to ensure its stability.

C. The program card must be inserted or removed only when the power switch is set to OFF. If the card is inserted when the power is ON, the card can be permanently damaged.

VI. Limitations of Procedure:

A. The DCA 2000+ HbA_{1C} assay will give accurate results when the total hemoglobin range is within 7 - 24 g/dl. However, if clinical conditions are present that push the hemoglobin value outside this range, the HbA_{1C} this assay should not be used. Such condition can include, severe anemias, polycythemia, homozygous HbS, and HbC.

B. Highly lipemic blood samples stored for long periods of time and/or frozen should be assayed with a different method.

VII. References:

A. Bayer Corporation, 1997. DCA 2000+ Analyzer, Operating Manual. Bayer Corporation, Elkhart, IN

B. Bayer Corporation, 1998. DCA 2000+ Hemoglobin A_{1C} package inserts

C. Hiar, Charles, April 1998. AClinical Performance of the DCA 2000+ Hemoglobin A_{1C} System Six-Minute Assay.@ Bayer Diagnostics Division, Tarrytown, NY

VIII. Hemoglobin A1c Procedure Review:

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Date								
Initials								

Date installed or replaced ____/____/____ Date removed _____/ ____/____

Supervisor: _____ Director: _____